International Journal of Agricultural Science and Research (IJASR)
ISSN(P): 2250-0057; ISSN(E): 2321-0087
Vol. 5, Issue 4, Aug 2015, 131-138

MORPHOLOGICAL AND PATHOGENICITY ASSAY OF *COLLETOTRICHUM GLOEOSPORIOIDES* – AN ANTHRACNOSE CAUSING PATHOGEN OF FRUITS AND VEGETABLES

MEENAKSHI SHARMA[1] & SAURABH KULSHRESTHA[2]

[1]Faculty of Biotechnology, Shoolini University of Biotechnology and Management Sciences, Bajhol, Solan, Himachal Pradesh, India

[2]Associate Professor, Faculty of Biotechnology, Shoolini University of Biotechnology and Management Sciences, Bajhol, Solan, Himachal Pradesh, India

ABSTRACT

Colletotrichum gloeosporioides is an anthracnose causing fungal pathogen of various fruits and vegetables and causes both pre and post harvest losses to crops leading to huge losses to farmers. In order to check the prevalence of *C. gloeosporioides* infecting fruit crops (Apple, Guava, Kiwi, and mango) of Himachal Pradesh, it was isolated and checked for morphological variations and pathogenicity. The guava isolate of Dhaula kuan showed same morphology in term of growth and sporulation with standard strain of guava procured from IMTECH Chandigarh. The kiwi isolate of Solan and mango isolate of Dhaula Kuan showed same morphology in term of growth and sporulation with each other. But apple isolate of Shimla was different in morphology (slow growth and less number of spores) from other isolates. Oat meal agar media was found to be best media for sporulation of *C. gloeosporioides* out of other tested media such as: Potato dextrose agar, Lima bean agar, Malt extract agar. Pathogenesis assay of *C. gloeosporioides* (standard isolate from IMTECH, Chandigarh) was done to check the virulence. The isolate was found to be pathogenic to all the fruits and their detached leaves tested.

KEYWORDS: *Colletotrichum gloeosporioides*, Anthracnose, Morphology, Pathogenesis, Media

INTRODUCTION

C. gloeosporioides proposed for the first time as *Vermicularia gloeosporioides* (Penzig, 1882) is now widely distributed and common plant pathogen in the world (Sutton, 1992; Cannon *et al*., 2002). It was found to be more abundant in tropical and subtropical regions than in temperate (cab international 2005). It infects about 470 different host genera and causes both pre and post-harvest problems (Prusky *et al*., 1992) and also act as endophytic strains which was isolated from symptomless plant parts (Cannon *et al*., 2002; Lu *et al*., 2004; Photita *et al*., 2004; Photita *et al*., 2005). Von Arx (1957) reported more than 600 synonyms of *C. gloeosporioides* showed many morphological and physiological variations. Palo (1932) described the morphology of the fungus and found that the spores were irregular and appear as brown to black dots. The acervuli were highly variable in size, shape and exude pink masses of conidia when mature under moist conditions (Sattar *et al*., 1939).

Anthracnose is a disease of the foliage, stems, or fruits that typically appear as dark-coloured spots or sunken lesions with a slightly raised rim (www.infonet-biodivision.org). *C. gloeosporioides* causes anthracnose disease on a wide variety of fruits, including almond, avocado, apple, Arabica coffee, guava, mango, strawberry, papaya, banana, passion

fruit, citrus, grapes and cashews (Simmonds, 1965; Hartill, 1992; Alahakoon *et al*., 1994; Timmer *et al*., 1998; Agwana *et al*., 1997; Freeman *et al*., 1998; Martínez- Culebras *et al*., 2003; Sanders and Korsten, 2003; Xiao *et al*., 2004; Amusa *et al*., 2005; Nelson, 2008). This disease is favored by wet, humid, warm conditions and spread by infected seeds, rain splash and moist winds. In this disease lesion appears on stolon and petioles. With age these lesions become dark and finally produced concentric ring pattern (Ponte, 1996; *infonet-biovision.com*).

C. gloeosporioides involves hemibiotrophic mode of infection where both biotrophic and necrotrophic phases occur sequentially. In biotrophic phase, infection vesicles and primary hyphae are formed and secondary hyphae developed and spread to kill the host cell in necrotrophic phase.

C. gloeosporioides requires optimum temperature of 25-28°C, pH range of 5.8-6.5 and high humidity for better growth. This pathogen is inactive in dry season and switches to active stages when encountered favorable environmental conditions.

From Himachal Pradesh, *C. gloeosporioides* was first reported from bell pepper (capsicum annuum) in 2005, 2006 (Gupta *et al.,* 2009) and has not been reported from other fruit and vegetable crops. In the present investigation an attempt was made to know the prevalence and morphological variations of *C. gloeosporioides* in various fruits. Pathogenicity of guava isolate of *C. gloeosporioides* was also studied to access its potential of causing symptoms to different crops and fruits.

MATERIALS AND METHODS

a) Isolation, Purification and Maintenance of Culture: Isolation of *C. gloeosporioides* was done from collected leaves samples of various orchids of H.P. by the method described by Choi *et al*., (1992). The collected leaves were washed and cut down into small pieces. Sterilization was done using 0.1% mercuric chloride and washed with autoclaved distilled water for 2-3 times (5 min each). Then sterilized leaves were wounded with scalpel blade and placed on pre poured PDA plates and incubation done at 25°C for 3-4 days. The fungal colonies which looked like *C. gloeosporioides* were transferred to fresh PDA plates. All cultures were single spored to get pure colony by sub culturing the spores on PDA plates and maintained as glycerol stock in -80°C freezer.

b) Morphological and sporuLation Study on Different Media: For morphological study, PDA media was used and sporulation of *C. gloeosporioides* was tested on four different media such as: PDA (Potato dextrose agar) (39g potato dextrose agar in 1000ml distilled water); Oat meal agar (30 g powdered oat meal, 20g agar, 1000ml distilled water); malt extract agar (30g malt extract, 5.0g mycological peptone, 15g agar, 1000ml distilled water); lima bean agar (23g lima bean agar in 1000ml distilled water). These media were prepared and sterilized and plates were prepared. For each experiment, four petridishes were taken as replicates. The inoculation on each media was done using 15days old culture of *C. gloeosporioides*. After inoculation, all plates were incubated at 25°C for 4-5 days. Spores were counted under microscope using haemocytometer. Photographs were taken and analyzed for both experiments.

c) Pathogenesis Assay: Pathogenesis assay was done on various detached leaves and fruits. The *C. gloeosporioides* was inoculated on oat meal agar media using 15 days old culture of *C. gloeosporioides* and plates were incubated at 25°C for 4-5 days. Spores were harvested in autoclaved water. The spore suspension of 10^6spores/ml was prepared using haemocytometer. 10µl of spore suspension was used to inoculate detached leaves and fruits.

The inoculated samples were incubated in humid box at room temperature. Inoculated samples were observed periodically for the development of symptoms and photographs were taken.

RESULTS

Four *Colletotrichum gloeosporioides* isolates were successfully isolated from leaf samples of Guava collected form Dhaula Kuan (Figure 1a); Mango leaves collected from Dhaula Kuan (Figure 1b); Kiwi leaves collected from Solan (Figure 1c); Apple leaves collected from Shimla (Figure 1d). Guava isolate of *C. gloeosporioides* (MTCC No. 4618) was also procured from IMTECH Chandigarh and was included as control in various experiments.

- **Morphological Study:** The guava isolate of Dhaula kuan (Figure 1a) showed same morphology in term of growth and sporulation with standard strain of guava procured from IMTECH Chandigarh (Figure 1e). The kiwi isolate of Solan (Figure 1c) and mango isolate (Figure 1b) of Dhaula Kuan were showed same morphology in term of growth and sporulation with each other. But apple isolate of Shimla (Figure 1d) was different in morphology from other isolates. The growth and number of spores where were very less as compared to other isolates.

Figure 1: Morphology of Different Collected Isolates on PDA Plates a) Guava Isolate from Dhaula Kuan b) Mango Isolate from Dhaula Kuan c) Kiwi Isolate from Solan d) Apple Isolate from Shimla e) Standard Guava Strain from IMTECH Chandigarh

- **Sporulation Study:** Sporulation study was conducted using various media reported for growth and sporulation of *C. gloeosporioides*. The maximum sporulation of *C. gloeosporioides* (standard strain MTCC 4618) was obtained on oat meal agar (Figure 2d) as compared to PDA (potato dextrose agar) (Figure 2a), malt extract agar (Figure 2b) and lima bean agar (Figure 2c). Similar results were also observed for all the isolates collected from various crops (data not shown).

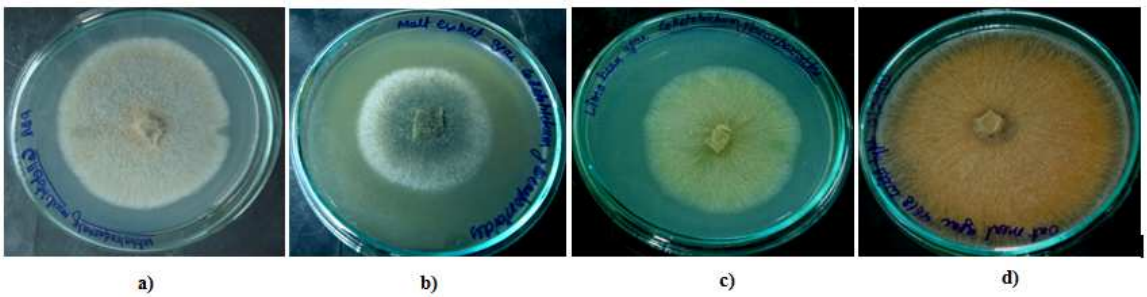

Figure 2: Sporulation Study *Colletotrichum gloeosporioides* Standard Strain on Different Media Plates a) PDA b) Malt Extract Agar c) Lima Bean Agar d) Oat Meal Agar

- **Pathogenesis Assay:** Pathogenicity assay was performed on various fruits namely apple, banana, orange, guava and on the detached leaves of various fruits namely apple, guava, kiwi, mango and peach. All inoculated leaves

and fruit pieces showed grey, black necrotic lesions along with aerial growth after 5-7 days of inoculation. Maximum pathogenicity was observed in case of kiwi leaf (Figure 3c) followed by apple leaf (Figure 3a), peach leaf (Figure 3e), guava leaf (Figure 3b) and minimum pathogenicity was observed in case of mango leaf (Figure 3d). In case of pathogenicity on fruits, maximum pathogenicity in terms of necrotic lesion on the surface and aerial growth of mycelium was observed on banana both on outer and inner surface (Figure 4b; Figure 5b), followed by orange (Figure 4c; Figure 5c) and apple (Figure 4a; Figure 5a). Minimum pathogenicity was observed in case of guava (Figure 4d; Figure 5d).

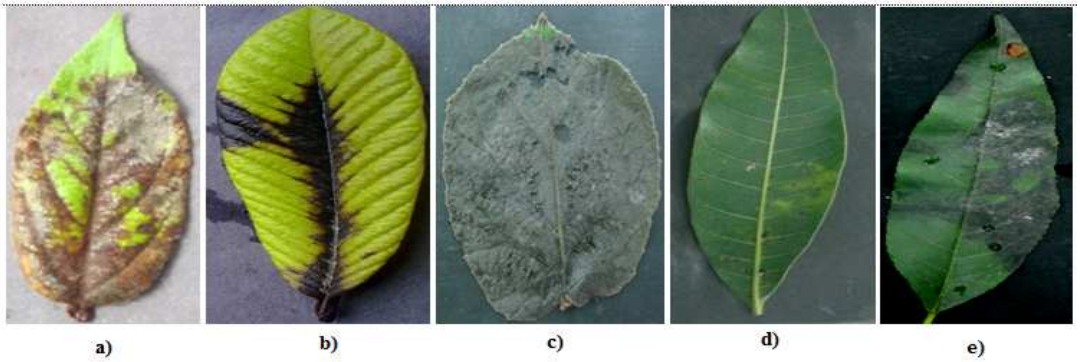

Figure 3: Pathogenesis Assay of *C. gloeosporioides* on Various Leaves a) Apple b) Guava c) Kiwi d) Mango e) Peach

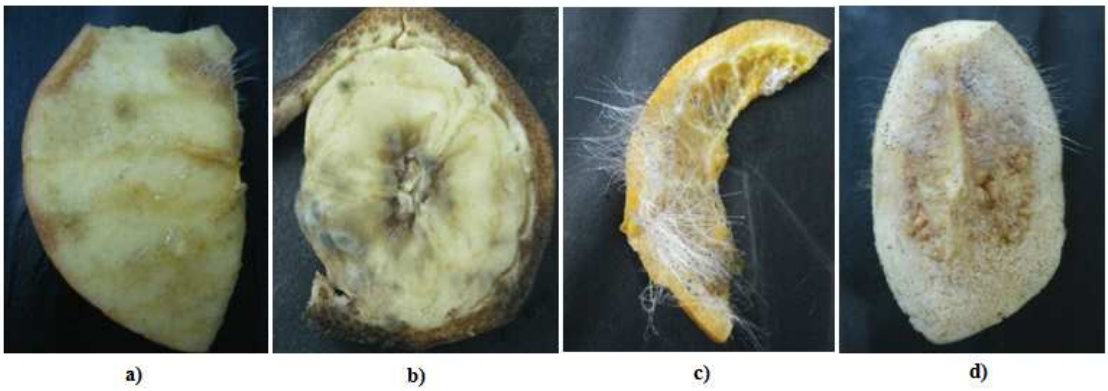

Figure 4: Pathogenesis Assay of *C. gloeosporioides* on various Fruit Pieces a) Apple b) Banana c) Orange d) Guava

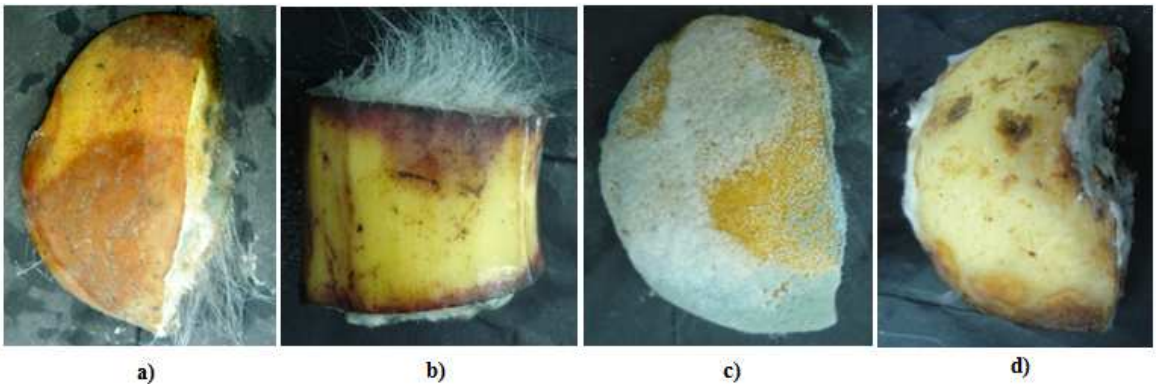

Figure 5: Pathogenesis Assay of *C. gloeosporioides* on Various Fruit Pieces a) Apple b) Banana c) Orange d) Guava

DISCUSSIONS

The morphological studies of *C. gloeosporioides* were also performed earlier by many researchers. Similar to the present investigation Zivkovic *et al*., (2010) performed morphological studies on different isolates of *C. gloeosporioides* by inoculating them on PDA (Potato dextrose agar) and has observed variation in the morphology of collected *C. gloeospotioides* which was also observed in the present investigation. The length and width of 100 conidia were measured and conidial shape was observed under light and scanning microscope. They used Slide culture technique to produce appressorium. After 5 days, the shape and size of the 100 appressorium formed across the underside of the coverslip were examined microscopically. Morphological characteristics of conidia and appressorium of tomato isolates were compared with reference isolates of *C. acutatum* and *C. gloeosporioides*. Grahovac *et al*., (2012) studied different isolates of *C. gloeosporioides* by inoculating them on PDA. Morphological study was done with various parameters such as shape, color, margin and conidial shape observed under light microscope. We have only used PDA for morphological study and it was performed on the basis of growth rate, colony colour and sporulation. Except for apple isolate from Shimla, which showed very slow growth and sporulation other isolates showed similar morphology in group of two (isolate from guava was similar to standard isolate and isolate from kiwi was similar to mango isolate).

The sporulation study of *C. gloeosporioides* and other plant pathogenic fungi using various media and effect of environmental factors such as temperature, light and pH were previously reported. V-8 juice agar and Richard's agar were found to be best media for growth and sporulation of *C. gloeosporioides*, out of several medias tested such as: potato dextrose agar, malt extract agar, oat meal agar, host leaf extract agar, corn meal agar, potato carrot agar, papaya fruit agar, V-8 juice agar, Richard's agar, Czapek's agar, Sabouraud's agar, Elliott's agar, Tochinal's agar, Sach's agar and Brown's agar (Tasiwal and Benagi, 2009). In different line, we have found that oatmeal agar has performed best for sporulation of *C. gloeosporioides* in comparison to other medias tested like PDA, malt extract agar and lima bean agar. Our results were in line with the results obtained by Mello *et al.*, (2004), they have also found that Oat media was best media for sporulation of *C. gloeosporioides* out of four media such as: PDA, oat, filtered pepper extract, and autoclaved pepper extract. Higher sporulation was observed when incubation was done in constant light (Mello *et al*., 2004). Growth and sporulation of *C. gloeosp orioides* was also done by Soltani *et al*., (2014) using four different medias such as: PCA (potato carrot agar), PDA (potato dextrose agar), WA (water agar), CLA (carnation leaf agar) and also studied the effect of light and temperature on the growth and sporulation. It has been observed that maximum growth was observed on PDA, PCA at 28°C temperature and continuous light conditions. Best sporulation was observed on fabriano paper placed on PDA media and 16/8 hrs light/darkness interval (Soltani *et al*., 2014). Inclusion of carbon source (fructose/starch/ xyllose), nitrogen source (asparatic acid/ potassium nitrate) and pH 5-6.5 of the growth media was also found to be the best for growth and sporulation of *C. gloeosporioides* (Kumara and Rawal, 2008; Deshmukh *et al*., 2012).

Pathogenicity assay revealed that *C. gloeosporioides* standard strain (MTCC No. 4618) and other isolated strains are capable of inducing symptoms on variety of fruits and their detached leaves with significant differences in the extent of infection obtained. Gupta *et al.*, (2010) performed Pathogenicity tests on the mango seedlings of Desheri under greenhouse condition using 25 *C. gloeosporioides* isolates. Spore suspension was inoculated by using pin prick inoculation technique. Three replicates were used for each isolates of *C. gloeosporioides*. During assay, the temperature was maintained from 15 to 30°C. At the end it was observed that all isolates were pathogenic to mango and caused 85 - 90% mortality in 2 - 3 months (Gupta *et al.*, 2010). In our pathogenic experiment, it was observed that all isolates of *C. gloeosporioides* are

pathogenic and disease symptoms were observed on various detached leaves. Talhinhas *et al*., (2005) performed pathogenicity test of *C. gloeosporioides* by using conidial suspension of 10^5 conidia· cm^{-3} containing 1% gelatin to inoculate strawberry and olive. The inoculated fruits were incubated in 100% relative humidity at room temperature. Symptoms were observed periodically. The pathogen showed necrotic lesions on petioles, stems and leaves of all inoculated fruits (Talhinhas *et al*., 2005). Hwang *et al*., (1995) generated random mutants of *C. gloeosporioides* and assessed their pathogenicity on avocado and tomato. During infection assay each fruit was inoculated with 5 drops (2000 conidia per 20µl per drop) of conidial suspension (Hwang *et al*., 1995). However, we have used 10000 conidia (1000x 10 µl droplet) for pathogenicity assay and symptoms were observed and photograph was taken after 11 days.

It was concluded from this study that *C. gloeosporioides* is a major pathogen infecting apple, guava, mango and kiwi in Himachal Pradesh and there is substantial variation amongst *C. gloeosporioides* isolates. These *C. gloeosporioides* isolates are also capable of infecting other crops. There is a need to conduct more comprehensive survey and molecular basis of variability amongst *C. gloeosporioides* isolates should also be looked into thoroughly in future.

ACKNOWLEDGEMENTS

The authors would like to thank Prof. P. K. Khosla, Hon'ble Vice-Chancellor, Shoolini University of Biotechnology and Management Sciences, Solan and Foundation for Life Sciences and Business Management (FLSBM), Solan for providing financial support and necessary facilities.

REFERENCES

1. Penzig, A.G.O. (1882). Fungi agrumicoli. Contribuzione allo studio dei funghi parassiti degli agrumi/ Michelia, 2, 385–508.
2. Sutton, B.C. (1992). The genus *Glomerella* and its anamorph *Colletotrichum*. In *Colletotrichum*: biology, pathology and control (eds. J.A.Bailey and M.J.Jeger)/ CAB International: Wallingford, 1-26.
3. Cannon, P.F., & Simmons, C.M. (2002). Diversity and host preference of leaf endophytic fungi in the Iwokrama Forest Reserve, Guyana/ Mycologia, 94, 210-220.
4. CAB International, Crop protection Compendium, 2005 Edition. Wallingford, U.K: CAB International. WWW.cabicompendium.org/cpc.
5. Prusky, D., & Plumbley, R.A. (1992). (Quiescent Infections of *Colletotrichum* in Tropical and Subtropical Fruits. In: Bailey JA, Jeger MJ, editors. *Colletotrichum*: Biology, Pathology, and Control. Wallingford: CAB International, 289-307.
6. Lu, G.Z., Cannon, P.F., Reid, A., & Simmons, C.M. (2004). Diversity and molecular relationships of endophytic *Colletotrichum* isolates from the Iwokrama Forest Reserve, Guyana/ Mycological Research, 108, 53-63.
7. Photita, W., Lumyong, S., Lumyong, P., Mckenzie, E.H.C., & Hyde, K.D. (2004). Are some endophytes of *Musa acuminate* latent pathogens/ Fungal Diversity, 16, 131-140.
8. Photita, W., Taylor, P., Ford, R.W.J., Hyde, K.D., & Lumyong, S. (2005). Morphological and molecular characterization of *Colletotrichum* species from herbaceous plants in Thailand/ Fungal Diversity, 18, 117-133.
9. Von Arx, J.A. (1957). Die Arten der Gattung *Colletotrichum* Cda/ Phytopathology Zeitschrift, 29, 414-468.

10. Palo, M.A. (1932). *Sclerotium* seed rot and seedling stem rot of mango/ The Philippines Journal Science, 52(3), 237-261.
11. Sattar, A., & Malik, S.A. (1939). Some studies on anthracnose of mango caused by *Glomerella cingulata* (Stonem.) Spauld. Sch. (*Colletotrichum gloeosporioides* Penz.)/ India Journal of Agriculture Science, 1, 511-521.
12. www.infonet-biovision.org.
13. www.infonet-biodivision.com.
14. Simmonds, J.H. (1965). A study of the species of *Colletotrichum* causing ripe fruit rots in Queensland/ Queensland journal of agriculture and animal science, 22, 437-459.
15. Hartill, W.F.T. (1992). Post-harvest rots of avocado in New Zealand and their control/ Brighton crop protection conference, 1157-67.
16. Alahakoon, P.W., & Brown, A.E. (1994). Host range of *Colletotrichum gloeosporioides* on tropical fruit crops in Sri Lanka/ International journal of pest management, 40(1), 23-26.
17. Timmer, L.W., Brown, G.E., & Zitko, S.E. (1998). The role of *colletotrichum spp*. In post harvest anthracnose of citrus and survival of *C. acutatum* on fruit/ Plant disease, 82.
18. Agwana, C.O., Lashermes, P., Trouslot, P., Combes, M., & Charrier. A. (1997). Identification of RAPD markers for resistance to coffee berry disease, *Colletotrichum kahawae*, in arabica coffee/ Euphytica, 97, 241-248.
19. Freeman, S., Katan, T., & Shabi, E. (1998). Characterization of *Colletotrichum* species responsible for anthracnose diseases of various fruits/ Plant disease, 82, 596-605.
20. Martinez-Culebras, P.V., Querol, A., Suarez-Fernandez, M.B., Garcia-Lopez, M.D., & Barrio, E. (2003). Phylogenetic relationships among *Colletotrichum* pathogens of strawberry and design of PCR primers for their identification/ Journal of phytopathology, 151, 135–174.
21. Sanders, G.M., & Korsten, L. (2003). Comparison of cross inoculation potential of South African avocado and mango isolates of *C. gloeosporioides*/ Microbiological research, 158, 143-150.
22. Xiao, C.L., Mackenzie, S.J., & Legard, D.E. (2004). Genetic and Pathogenic Analyses of *Colletotrichum gloeosporioides* Isolates from strawberry and non cultivated hosts/ Phytopathology, 94(5), 446-53.
23. Amusa, N.A., Ashaye, A.O., Oladapo, M.O., & Oni, M.O. (2005). Guava fruit anthracnose and the effects on its nutritional and market values in Ibadan, Nigeria/ World Journal of agriculture sciences, 1, 169-172.
24. Nelson, S.C. (2008). Mango anthracnose (*Colletotrichum gloeosporioides*)/ Plant disease, 48.
25. Ponte, J.J. (1996). da. Clinica de doencas de plantas/ Fortaleza-CE: UFC, 871.
26. Gupta, S.K., Jarial, K., & Kansal, S. (2009). *Colletotrichum gloeosporioides* causing anthracnose in bell pepper seed crop: a new record from Himachal Pradesh/ Journal of plant disease sciences, 4(1), 126-127.

27. Choi, G.H., Larson, T.G., & Nuss, D.L. (1992). Molecular analysis of the laccase gene from the chestnut blight fungus and selective suppression of its expression in an isogenic hypovirulent strain/ Molecular plant-microbial interactions, 5, 119–128.

28. Zivkovic, S., Sasa, S., Ivanovic, Z., Trkulja, N., Dolovac, N., Aleksic, G., & Balaz, J. (2010). Morphological and molecular identification of *Colletotrichum acutatum* from tomato fruit/ The Journal of pesticides and phytomedicine, 25(3), 231-239.

29. Grahovac, M., Indic, D., Vukovic, S., Hrustc, J., Gvozdenac, S., Mihajlovic, M., & Tanovic, B. (2012). Morphological and ecological features as differentiation criteria for *Colletotrichum* species/ Zemdirbyste agriculture, 99(2), 189-196.

30. Tasiwal, V., & Benagi, V.I. (2009). Studies on the cultural and nutritional characteristics of *Colletotrichum gloeosporioides*, the causal organism of papaya anthracnose/ Karnataka journal of agriculture sciences, 22(4), 787-789.

31. Mello, A.F.S., Machado, A.C.Z., & and Benendo, I.P. (2004). Development of *Colletotrichum gloeosporioides* isolated from green pepper in different culture media, temperature and light regimes/ Scientia agricola (Piracicaba, Braz.), 61(5), 542-544.

32. Soltani, J., Haghighi, M.Y.P., & Nazeri, S. (2014). Light, temperature, aging dependent vegetative growth and sporulation of *Colletotrichum gloeosporioides* on different culture media/ Journal of medicinal plants research, 8(4), 208-216.

33. Kumara, K.L.W., & Rawal, R.D. (2008). Influence of carbon, nitrogen, temperature and pH on the growth and sporulation of some Indian isolates of *Colletotrichum gloeosporioides* causing anthracnose disease of papaya (Carrica Papaya L)/ Tropical agricultural research and extension, 11, 7-12.

34. Deshmukh, A.J., Mehta, B.P., Sabalpara, A.N., & Patil, V.A. (2012). In vitro effect of various nitrogen, carbon sources and pH regimes on the growth and sporulation of *Colletotrichum gloeosporioides* Penz. and Sacc causing anthracnose of Indian bean/ Journal of biopest, 5 (supplementary), 46-49.

35. Gupta, V.K., Pandey, A., P Kumar, P., Pandey, B.K., Gaur, R.K., Bajpai, V., Sharma, N., & Sharma, S. (2010). Genetic characterization of mango anthracnose pathogen *C. gloeosporioides* Penz. by random amplified polymorphic DNA analysis/ African journal of biotechnology, 9(26), 4009-4013.

36. Talhinhas, P., Sreenivasaprasad, S., Martins, J.N., & Oliveira, H. (2005). Molecular and phenotypic analyses reveal association of diverse *Colletotrichum acutatum* groups and a low level of *C. gloeosporioides* with olive anthracnose/ Journal of applied and environmental microbiology, 71(6), 2987-2998.

37. Hwang, C.S., Flaishamn, M.A., & Kolattukudy, P.E. (1995). Cloning of a gene expressed during appressorium formation by *Colletotrichum gloeosporioides* and a marked decrease in virulence by disruption of this gene/ Plant cell, 7, 183-193.